PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
992365woMekk		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/08023	22/10/1999	23/10/1998
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant NITSCH, ROGER et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14/04/2000	18.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hoesel, H Telephone No. +49 89 2399 8693

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08023

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-39 as originally filed

Claims, No.:

1-36 with telefax of 30/11/2000

Drawings, sheets:

1/10-10/10 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 28 - 36.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 28 - 36.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1 - 27.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1 - 27
	No:	Claims
Inventive step (IS)	Yes:	Claims 1 - 27
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1 - 27
	No:	Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: PETURSDOTTIR, I et al: 1st Symp. on Hereditary CAA, Reikjavik, 1985. & Acta Neurol Scand vol. 73/3, (1986), p. 318.
D2: Olafson\et al, Scan. J. Lab. Invest. vol. 48, 1988, p. 573 - 582

SECTION IV:

1. The application lacks unity within the meaning of Rule 13 PCT for the following reasons:

Claims 1 - 27 relate to a method of diagnosing Alzheimer's Disease ("AD") using cystatin C ("CC") as diagnostic marker. Correlation of AD with increased levels and polymorphisms in the CC gene have been demonstrated.

Claims 28 - 36 relate to compounds changing the activity of CC, screening methods to identify such compounds and their medical use.

The common link between this group is established by CC itself, which compound is known in the prior art as are its use as marker, its physiological substrates, and antibodies specific therewith.

This common concept is thus neither novel nor inventive, contrary to Rule 13(2) PCT.

Thus, the above-mentioned groups of inventions are not so linked as to form a single general inventive concept (Rule 13.1 PCT).

SECTION V:

2. The state of the art referred to in the international search report does not disclose a method of prognosing or diagnosing AD (Alzheimer's disease) by determination level or activity in CSF of CC or determination of the CST-3 genotype of the individual. It also appears that test kits have not been disclosed in the prior art.

The subject-matter of claims 1 - 25 is therefore considered to be novel.

3. The relevant prior art for the present method results from D1 and the Davidsson publication (hereinafter cited as D3) referred to on p. 4, line 21 - p. 5, line 2 of the description.

D1, which has been published in 1985, discloses the measurement of the CC level in CSF of Down syndrome patients and a normal control group. It is suggested that similar tests should be performed on patients suffering from AD. This abstract appears prima facie to contain a motivation to start with such tests falling within the terms of present claims one and 4 - 10.

It can however be agreed with the applicant's argument that a skilled person would not pursue the suggestions given in D1 in view of later work presented in D3 according to which no correlation of the CSF levels of CC with AD could be shown.

Thus the prior art of D3, which is considered to represent the closest prior art for the claimed method with regard to the content and the time of publication, teaches away from the present subject-matter.

The prior art as taken into consideration is silent as to a correlation of level and /or activity of CC in CSF and the progression of AD or the efficacy of a treatment of AD. The prior art does, furthermore, not suggest a correlation between the expression of particular CST-3 alleles and AD.

Consequently, the method of of claims 1 - 16 is considered to be novel and inventive in the sense of Articles 33(2) and (3) PCT. The same applies to the use of a kit as defined in claims 17 - 22.

4. Kits suitable for the diagnosis of AD that contain reagents that selectively detect presence or absence of a polymorphism in the CC gene are neither disclosed nor suggested in the prior art taken into consideration.

Under that presumption that these kits do no include reagents that are useful to

detect a polymorphism at codon 68 (cf. claims 23 subsection (c)), the subject-matter of claims 23 - 27 is regarded as novel and inventive in the sense of Art. 33(2) and (3) PCT

SECTION VIII:

5. According to the description correlations have been shown for the level/activity of CC in CSF only but not in any other body fluid. Independent claims 1 - 3 which extend to assays of CC in body fluids other than CSF are therefore not supported by the actual disclosure in this respect Art.(6 PCT).

6. Claim 3 does not meet the requirements of Art. 6 PCT, as it is not substantially supported.

The application fails to demonstrate a significance of CC as a marker for the evaluation of a therapeutical success. In the absence of a therapy for AD at the effective date of the present application, the use application of CC as marker for the success of a therapy appears to be of hypothetical nature only.

It is, moreover, indicated in the application that an increase of CC expression/ activity is temporary and characteristic for early AD stages (see the paragraph extending between P35 and 36). Thus, "normalization" of the CC level/activity in a patient subjected to an AD therapy is not necessarily indicative for the success or efficiency of a treatment but may also indicate the procession of the disease, i.e. the failure of the treatment.

It is similarly not evident how a conventional drug therapy could alter the genetic pattern of a patient expressing one or two CST-3 B alleles.

7. The objection as lack of support (Art. 6 PCT) of claims 1, 13 and 14 is maintained in spite of the applicant's arguments

It is noted that in the present specification only one particular allele ("CST-3 B") located in the 5' flanking region of the CSF-3 gene, the existence of which was known (p. 2, lines 5 - 14), has been shown to correlate with elevated CC levels in

CSF. With respect to the location of the polymorphism, the primary sequence of the expression product is not altered.

At present claims 1, 13 and 14 cover the diagnostic use of a variety of "silent" polymorphisms that are not accompanied by an increased risk of AD (such as another known polymorphism in codon 68 of the CST-3 gene) or which are not correlated with a change of phenotype as they are neither translated nor alter the sequence of important regulatory elements of the CSF-3 gene.

Although it cannot be excluded that other nucleic acid alterations that are linked with the CST-3 allele A/B locus cannot be excluded, the application fails to identify any one of the postulated linkages.

Insofar as such embodiments are encompassed, the said claims are considered to lack essential technical details and not to be reproducible over their entire field (Art. 6 PCT and 5 PCT).

8. Claim 17 does not meet the requirements of Art. 6 PCT for the following reasons:

(i) The claim contains a variety of redundancies and contradictions concerning the evaluation of the method (step (b)), for instance, mentions on one hand evaluation of "varied CC levels" and on the other of "increased levels".

(ii) The alternative "(wherein) a level, activity or both of ... being similar or equal to a reference value representing a known disease status... (indicates...)" makes no technical sense unless the disease status is clarified to be AD.

9. Claim 24 lacks clarity for the following reasons (Art. 6 PCT):

(i) The claim contains the same redundancies and contradictions as discussed above in conjunction with claim 17.

(ii) Attention is additionally drawn to the fact that features relating to a method are usually technically meaningless as a characterization of a product to which claim 24. Thus the features given under subsection (b) of are either irrelevant and

superfluous, or, if they are intended as a limitation, raise uncertainty as to the scope and category of the claim.

10. It can be derived from the wording of subsection (b) of claim 23, that the claimed kit essentially comprises reagents that selectively detect the presence or absence of the B allele of the CC gene. The component (a) of the kit should have been clarified in this respect.

CLAIMS

1. A method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:

determining a level, or an activity, or both said level and said activity, of a transcription product and/or a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene in a sample from said subject;

and

comparing said level, or said activity, or both said level and said activity, of said transcription product and/or said translation product to a reference value representing a known disease or health status,

thereby diagnosing or prognosing Alzheimer's disease in said subject, or determining whether said subject is at increased risk of developing Alzheimer's disease.

2. A method of monitoring the progression of Alzheimer's disease in a subject, comprising:

determining a level, or an activity, or both said level and said activity, of a transcription product and/or a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene in a sample from said subject;

and

comparing said level, or said activity, or both said level and said activity, of said transcription product and/or said translation product to a reference value representing a known disease or health status,

thereby monitoring the progression of Alzheimer's disease in said subject.

3. A method of evaluating a treatment for Alzheimer's disease, comprising:

determining a level, or an activity, or both said level and said activity, of a transcription product and/or a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene in a sample obtained from a subject being treated for Alzheimer's disease;

and

comparing said level, or said activity, or both said level and said activity, of said transcription product and/or said translation product to a reference value representing a known disease or health status,

thereby evaluating said treatment for Alzheimer's disease.

4. The method according to one of claims 1 to 3, wherein said sample is a body fluid, preferably cerebrospinal fluid.

5. The method according to claim 4, wherein an increase of a level or a varied activity of a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene in said cerebrospinal fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

6. The method according to one of claims 1 to 5, wherein said subject is a human.

7. The method according to one of claims 1 to 6, wherein said translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene is determined in its monomer form.

8. The method according to one of claims 1 to 7, wherein said translation product and/or said transcription product is detected using an immunoassay, an enzyme activity assay and/or a binding assay.

9. The method according to one of claims 1 to 8, wherein said reference value is that of a level, or an activity, or both said level and said activity, of a transcription product and/or a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene in a sample from a subject not suffering from said Alzheimer's disease.

10. The method according to one of claims 1 to 9, further comprising comparing a level, or an activity, or both said level and said activity, of a transcription product and/or a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene, in said sample with a level, an activity, or both said level and said activity, of a transcription product and/or a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene in a series of samples taken from said subject over a period of time.

11. The method according to claim 10, wherein said subject receives a treatment prior to one or more sample gatherings.

12. The method of claim 11, wherein said level, or said activity, or both said level and said activity, in said samples is determined, before and after said treatment of said subject.

13. A method of diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease comprising:

determining a presence or absence of a polymorphism in a cystatin C gene in a sample from said subject,

thereby diagnosing or prognosing Alzheimer's disease in said subject, or determining whether said subject is at increased risk of developing Alzheimer's disease.

14. The method of claim 13, wherein a presence of a polymorphism in leucin 68 codon of a human cystatin C gene leading to a loss of *Alu* I restriction site does not indicate diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

15. The method of claim 13 and/or 14, wherein the presence or absence of at least one B allele is determined.

16. The method of claim 15, wherein the presence of at least one B allele, in particular the presence of the B/B genotype, indicates said subject is at increased risk of developing Alzheimer's disease or indicates a diagnosis or prognosis of Alzheimer's disease.

17. The method of at least one of claims 13 to 16, further comprising:

determining a level, or an activity, or both said level and said activity, of a transcription product and/or a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene in a sample from said subject;

and

comparing said level, or said activity, or both said level and said activity, of said transcription product and/or said translation product to a reference value representing a known disease or health status.

18. A kit for diagnosis, or prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:

(a) at least one reagent which is selected from the group consisting of

- reagents that selectively detect a transcription product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene,
- reagents that selectively detect a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene, and
- reagents that selectively detect the presence or absence of a polymorphism in a cystatin C gene; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by
- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene, in a sample from said subject; and/or detecting a presence or absence of a polymorphism in said cystatin C gene in a sample from said subject; and
- diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,
wherein
a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status;
or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status;
or the presence of a polymorphism in said cystatin C gene indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

19. The kit according to claim 18, wherein a presence of a polymorphism in leucin 68 codon of a human cystatin C gene leading to a loss of *Alu* I restriction site does not indicate diagnosis or prognosis of Alzheimer's disease in said subject.

20. The kit according to one of claims 18 to 19, further comprising reagents to assess a function or dysfunction of said subject's kidneys.

21. The kit according to one of claims 18 to 20, wherein the presence of at least one B allele, in particular the presence of the B/B genotype, indicates a diagnosis, or prognosis, or an increased risk of development of Alzheimer's disease.

22. The kit according to at least one of claims 18 to 21, wherein an increase of a level or a varied activity of said translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene in said cerebrospinal fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

23. The kit according to one of claims 18 to 22, wherein said translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene is determined in its monomer form.

24. The kit according to at least one of claims 18 to 23 for use in monitoring a progression of Alzheimer's disease in a subject.

25. The kit according to at least one of claims 18 to 23 for use in monitoring success or failure of a therapeutic treatment of said subject.

26. A method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect an activity, or level, or both said activity and level, of

- a cystatin C gene or a polymorphic variant of a cystatin C gene, and/or
- a transcription product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene, and/or
- a translation product of (i) a cystatin C gene or (ii) a polymorphic variant of a cystatin C gene.

27. The method according to claim 26, wherein said agents are cathepsin derivatives or cystatin C analoga.

28. The method according to one of claims 25 to 27, wherein per se known methods of gene therapy and/or antisense nucleic acid technology are applied to administer said agent or agents.

29. The method according to at least one of claims 25 to 28 comprising grafting donor cells into the central nervous system, preferably the brain, of said subject, said subject or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents.

30. An agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a cystatin C gene, a polymorphic variant of a cystatin C gene, a transcription product of a cystatin C gene, a transcription product of a polymorphic variant of a cystatin C gene, a translation product of a cystatin C gene and a translation product of a polymorphic variant of a cystatin C gene.

31. A medicament comprising an agent according to claim 30.

32. An agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a cystatin C gene, a polymorphic variant of a cystatin C gene, a transcription product of a cystatin C gene, a transcription product of a polymorphic variant of a cystatin C gene, a translation product of a cystatin C gene and a translation product of a polymorphic variant of a cystatin C gene for treating or preventing a disease.

33. Use of an agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a cystatin C gene, a polymorphic variant of a cystatin C gene, a transcription product of a cystatin C gene, a transcription product of a polymorphic variant of a cystatin C gene, a translation product of a

cystatin C gene and a translation product of a polymorphic variant of a cystatin C gene for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular Alzheimer's disease.

34. A method for identifying an agent that directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a cystatin C gene, a polymorphic variant of a cystatin C gene, a transcription product of a cystatin C gene, a transcription product of a polymorphic variant of a cystatin C gene, a translation product of a cystatin C gene and a translation product of a polymorphic variant of a cystatin C gene, comprising the steps of:

(a) providing a sample comprising at least one substance which is selected from the group consisting of a cystatin C gene, a polymorphic variant of a cystatin C gene, a transcription product of a cystatin C gene, a transcription product of a polymorphic variant of a cystatin C gene, a translation product of a cystatin C gene and a translation product of a polymorphic variant of a cystatin C gene;

(b) contacting said sample with at least one agent;

(c) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.